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THE EFFECTS OF EXPERIMENTAL RESERVOIR CREATION ON THE BIOACCUMULATION OF MEHG AND REPRODUCTIVE SUCCESS OF TREE SWALLOWS (*Tachycineta bicolor*)

by

Pauline M. Gerrard

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

Environmental Biology and Ecology
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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **The effects of experimental reservoir creation on the bioaccumulation of methylmercury and reproductive success of tree swallows (*Tachycineta bicolor*)** submitted by **Pauline Marie Gerrard** in partial fulfillment of the requirements for the degree of Master of Science in Environmental Biology and Ecology.

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ABSTRACT

Reservoir creation results in decomposition of flooded organic matter and increased rates of mercury methylation. Methylmercury, the most toxic form of mercury, bioaccumulates through aquatic food webs. The objective of this study was to quantify transfer of methylmercury from aquatic food webs into terrestrial organisms. We addressed this objective by examining rates of methylmercury bioaccumulation in an insectivorous songbird, the tree swallow, breeding near an experimentally created reservoir. We also determined the impact of flooding and methylmercury bioaccumulation on the reproductive success of these birds. We found that MeHg burdens in nestling swallows from near the experimental reservoir were significantly greater postflood than prior to flooding. Although methylmercury burdens in swallows were elevated in postflood years, we found no overt toxicological affects. In fact, an increase in Dipteran productivity (the primary food source of tree swallows) in the reservoir because of flooding resulted in elevated reproductive success during postflood years.

I would like to dedicate this work to Mr. Downtown Rahj. Thursday mornings may never be the same again.

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INTRODUCTION

Mercury in the Environment

The anthropogenic release of mercury (Hg) into the environment is increasingly becoming a major concern globally. Mercury poisoning in humans was described as far back as early Chinese times, where it was thought that cinnabar, a mercury-containing mineral, could prolong life. Mercury was first mined in the Spanish Almaden over 2,000 years ago, and there, miners would eventually get sick and usually die (which was then thought acceptable because they were prisoners and slaves). The expression "mad as a hatter" comes from the twitching and dementia once common among hat makers, who dipped felt in mercuric nitrate to soften it. However, its potential effects on human populations as a whole became evident in the 1950s in Minimata, Japan. There, effluent containing high concentrations of inorganic mercury dumped into Minimata Bay from an industrial chemical manufacturer resulted in widespread mercury poisoning among the local fishing population (Kurland *et al.*, 1960). In the sediments of the Bay, inorganic Hg was converted to methylmercury (MeHg, organic mercury). Methylmercury is a strong neurotoxin that readily bioaccumulates up the food chain (Gilmour and Henry 1991; Kidd *et al.*, 1995; Mason *et al.*, 1995) and can reach toxic concentrations in upper trophic level organisms despite relatively low concentrations in water (Bodaly *et al.*, 1984a). Although Hg has a natural biogeochemical cycle, humans have dramatically increased sources to the atmosphere (Mason *et al.*, 1994). Increased anthropogenic sources of Hg include industrial activities such as coal combustion and solid waste incineration. It is commonly found in house hold items including batteries, florescent light bulbs, paints and solvents (Carp 1996).

The most common forms of mercury are elemental mercury (Hg^0) and divalent mercury (HgII) (Winfrey and Rudd 1990). Elemental mercury is volatile, only slightly water-soluble and undergoes long-range atmospheric transport. Divalent mercury is water-soluble and readily associates with inorganic and organic molecules. It is produced in the atmosphere through a series of photocatalitic reactions and is deposited as both wet and dry deposition (St. Louis *et al.*, 1995; Winfrey and Rudd 1990). Divalent mercury is methylated to MeHg through both biotic and abiotic processes (Carp 1996).

Biotically, methylation appears to be most commonly mediated by sulfate reducing bacteria (Compeau and Bartha 1985) in anaerobic environments such as the surface layer of lake sediments (Winfrey and Rudd 1990) or wetlands (St. Louis *et al.*, 1996). Environmental factors such as oxygen concentration, temperature, availability of biodegradable organic carbon, pH, DOC, and redox conditions play a large role in determining mercury methylation and bioavailabilty to the aquatic food chain (Berman and Bartha, 1986; Gilmour and Henry, 1991; Miskimmin *et al.*, 1992; Winfrey and Rudd 1990). Once produced, MeHg can be destroyed either microbially through demethylation (Ramlal *et al.*, 1986) or as a result of photodegradation (Sellers *et al.*, 1996). Methylmercury concentration in aquatic ecosystems is the net result of atmospheric deposition, microbial methylation and demethylation and photodegradation. The efficiency of each of these processes dictates MeHg bioavailability and bioaccumulation (Sellers *et al.*, 1996; St. Louis *et al.*, 1995; Winfrey and Rudd 1990). Abiotic methylation of Hg is rarer than biotic methylation, and usually requires environments with high concentrations of both inorganic Hg and organic carbon (Lee and Hultberg, 1990).

Mercury contamination in fish is a serious problem in both industrial regions and remote areas across Canada and the United States. For example, 70% of lakes in

Ontario have consumption advisories due to high levels of Hg in fish (OMOE, 1997). It is well understood that fish derive the majority of their MeHg not directly from the water they swim in, but from the food they consume (Hall *et al.*, 1997). Less is known about mercury uptake in lower food chain organisms, such as algae and invertebrates. Many aquatic invertebrates spend their larval stages living in aquatic sediments or in the water column, but emerge as winged adults to reproduce. These winged adults provide an important vector for nutrient flux from aquatic into terrestrial ecosystems, but can also result in fluxes of Hg (Bissonnette, 1975; Parkman and Meili, 1993). Insectivorous and piscivorous birds and mammals rely on aquatic ecosystems for food, and may be exposed to dietary MeHg concentrations that are high enough to cause physical, reproductive or behavioral impairment.

Methylmercury Toxicity

Methylmercury toxicity is primarily manifested in the central nervous system and includes sensory and motor deficits as well as behavioral impairment (Aulerich *et al.*, 1974; Wren *et al.*, *1988*). Methylmercury readily crosses the placenta as well as the blood brain barrier and has been shown to concentrate selectively in the fetal brain (Clarkson, 1987). Once MeHg has been ingested, it is adsorbed from the gastrointestinal tract and binds readily to sulf-hydryl groups in proteins. It enters the brain as an amino acid complex and disrupts protein synthesis (Clarkson, 1987). At high concentrations, damage occurs to the cerebellum and cerebrum with neuron necrosis, lyses and phagocytosis. Effects in mammals include ataxia, difficulty in locomotion, weakness, impairment of hearing and vision, tremor and loss of consciousness and death (Wolfe, 1998). At levels lower than those that cause acute toxicity, behavioral and reproductive

effects are common. In birds, overall reproductive success can decrease significantly with reductions in egg laying and nest-site fidelity (Heinz, 1979; Finley *et al.*, *1978*; Meyer *et al.*, 1998). Methylmercury readily binds to fur and feathers during growth and this becomes an important route of excretion in both birds and mammals. In many mammals, up to half of the body burden can be removed through hair growth (Clarkson, 1987).

Mercury in Birds

Considerable work has been done on mercury toxicity in birds. Studies were initiated because of the practice of treating seed with organomercurl fungicides beginning in the late 1940s, which resulted in widespread contamination of seed-eating birds and their predators (Scheuhammer, 1987; Fimreite, 1979). More recently there has been interest in birds as indicator species of heavy metal toxicity. In the 1970s, Fimreite found that wild bird species living in environments receiving little or no industrial Hg contamination had liver concentrations ranging from 1-10 μg/g wet weight (Fimreite, 1974) while species from contaminated areas had liver concentrations as high as 17.4 μg/g wet weight (Fimreite *et al.*, 1971). Seabirds have also commonly been used as bioindicators of Hg contamination. Estuarine and marine systems act as a sink for metals from continental sources and are often high in heavy metal concentrations (Bourne, 1976). There has been a wide range of research done on tissue Hg concentrations and burdens in seabirds, which has included their associated dietary items, molting strategies, and the chemical form of Hg stored in tissues (Kim *et al.*, 1996; Lock *et al.*, 1992; Thompson and Furness, 1989; Thompson *et al.*, 1990). Most recently, common loon (*Gavia immer*) populations in eastern Canada have been found to have exceedingly high blood and tissue Hg

concentrations despite the lack of an obvious point source for the pollutant (Burger, 1993; Evers *et al.*, 1998). Heavy metal concentrations in these top predatory species are currently being widely studied across the United States and Canada (Daoust *et al.*, 1998; Evers 1998; Meyer *et al.*, 1998; Nocera and Taylor, 1998; Pokras *et al.*, 1998).

Physiologically, absorption of ingested MeHg across the avian gut wall is >90% (Scheuhammer, 1987). As in mammals, MeHg readily crosses the blood brain barrier and at chronic levels spinal cord degeneration is characteristic. Clinical symptoms in birds include decreased food intake leading to weight loss, progressive weakness in wings and legs with difficulty flying, walking and standing (Borg *et al.*, 1969; Fimreite and Karstad, 1971). It has been suggested that neurological effects may be associated with liver Hg levels as low as 5 μg/g wet weight, although there are some long-lived species that can accumulated high concentrations with no apparent adverse affect (Kim *et al.*, 1996; Thompson and Furness 1989). Procellariifomes (albatrosses, petrels, and shearwaters) in particular have been found with very high Hg concentrations in their tissues and appear to be capable of demethylating MeHg in their livers. This liver demethylation process seems to be a species-specific response and has been hypothesized to be more common in species with long periods of time between molts (Thompson and Furness, 1989). The concentrations of MeHg required to affect reproduction is significantly lower than those required to produce other pathological effects. For example, reductions in egg laying and territorial fidelity in ducks have been associated with mean prey Hg concentrations as low as 0.3-0.4 μg/g wet weight (Barr, 1986; Heinz, 1979) and mean egg concentrations ranging from 0.5-1.5 μg/g (Fimreite *et al.*, 1971). Low levels of dietary MeHg have also been shown to cause reduced clutch

size, increased numbers of eggs laid outside the nest and aberrant behavior of juveniles (Wolfe, 1998).

The half-life of MeHg in many bird species has been estimated to be between 2-3 months (Scheuhammer, 1987). Species that undergo annual molts tend to have much lower body burdens than those who replace their feathers over a period of years (Thompson and Furness, 1989). The vast majority of Hg found in feathers is MeHg, which is deposited during molt, when feathers are actively growing and have a corresponding blood supply (Burger and Gochfeld, 1997; Thompson and Furness, 1989). MeHg binds to the sulfide bonds of feather keratin, and as a result, cannot be redistributed or removed through weathering (Appelquist *et al.*, 1984). Feathers can be used as an indication of mercury body burdens in birds, although time of last molt and feather type, as well as age and species of bird, must all be considered (Wolfe, 1998).

Mercury Associated with Reservoirs

Predatory fish in reservoirs that have been created for the production of hydroelectricity, recreation, flood control, or other purposes(ICOLD, 1988) almost always contain MeHg concentrations that far exceed guidelines for consumption (0.5 μg/g wet weight, for commercial sale in Canada; Health Canada) (Bodaly *et al.*, 1984a; Lodenius *et al.*, 1983). Methylation of inorganic mercury appears to be enhanced by the decomposition of organic carbon in flooded soils and vegetation (Hecky *et al.*, 1991). There is currently over 72,000 km^2 of hydroelectric reservoir surface area in Canada (St. Louis *et al.*, 2000). The creation of these reservoirs, and the subsequent bioaccumulation of MeHg in the fish, has greatly impacted the socioeconomic structure of first nation communities that rely on the fisheries for food (Bodaly *et al.*, 1984a; Rosenberg *et al.*, 1995). Other

environmental impacts of reservoir creation include the production of the greenhouse gases CO_2 and CH_4 from the decomposition of the flooded organic matter and reductions in biodiversity through habitat destruction(Bodaly *et al.*, 1984a; Rosenberg *et al.*, 1995).

The Experimental Lakes Area Reservoir Project (ELARP)

The ELARP is a multidisciplinary experiment designed to increase our knowledge of the environmental impacts of reservoir creation. In particular, the two primary objectives of the study were to improve our understanding of the biogeochemical cycling of mercury in reservoirs and to quantify greenhouse gas production as a result of flooding (Kelly *et al.*, 1997). The ELARP is a whole-ecosystem manipulation experiment that was carried out at the Experimental Lakes Area (ELA) in northwestern Ontario, beginning in 1991. In June of 1993, a small riverine wetland was flooded by closing a dam constructed at the outflow of the wetland, and raising the water level 1.3 meters. Each year the water level was drawn down in the fall and raised again in the spring to simulate water regimes maintained in many hydroelectric reservoirs. Intensive sampling was conducted during the first three years after flooding (Kelly *et al.*, 1997, Paterson *et al.*, 1998). Beginning in 1996 an alternate year sampling schedule was adopted and will be maintained indefinitely.

To address the first objective, the ELARP compared MeHg concentrations in water, zooplankton, benthic aquatic insects, omnivorous fish (finescale dace; *Phoxinus neogaeus*) and piscivorous fish (northern pike; *Esox lucius*) before flooding with those after. Initial findings of the ELARP included a profound change in MeHg production in the experimental reservoir after flooding with a subsequent increase in MeHg concentrations

in water and food chain organisms. The average percentage of total Hg (THg, all forms of Hg) that was MeHg in the surface waters of the experimental reservoir increased from on average 4%-32% after flooding (Kelly *et al.*, 1997). MeHg concentration in the water of the central pond averaged 0.1 ng/L before flooding and 0.9 ng/L during the first two years after flooding, but reached concentrations as high as 2.4 ng/L (Fig. 1). During the third year after initial flooding, MeHg concentrations in the water column began to decline slightly. However, in 1998, six years after initial flooding, MeHg concentrations in the water column were still significantly higher than those measured preflood (Fig. 1). Similarly, concentrations of MeHg in zooplankton from the experimental reservoir increased by 10 times within six weeks of initial flooding. Preflood concentrations of MeHg in zooplankton collected with an 80μm net ranged from 11-54 ng/g while post flood MeHg concentrations were often in excess of 300 ng/g (Paterson *et al.*, 1998). MeHg concentrations also increased after flooding by approximately 3 times in aquatic macroinvertabrate predators and 1-4 times in collector/shedder macroinvertabrates (Hall *et al.*, 1998). MeHg concentrations in yearling finescale dace living in the experimental reservoir were 0.10 ng/g before flooding and 0.32 ng/g after flooding. MeHg uptake by finescale dace increased by a factor of 3 times (Bodaly *et al.*, 1984b).

Objectives of this Study

Our objective as part of the ELARP was to quantify the transfer of MeHg from the aquatic food web into terrestrial organisms. We addressed this objective by examining long-term rates of MeHg bioaccumulation in an insectivorous songbird, the tree swallow (*Tachycineta bicolor*), attracted to nest and reproduce near the experimentally flooded

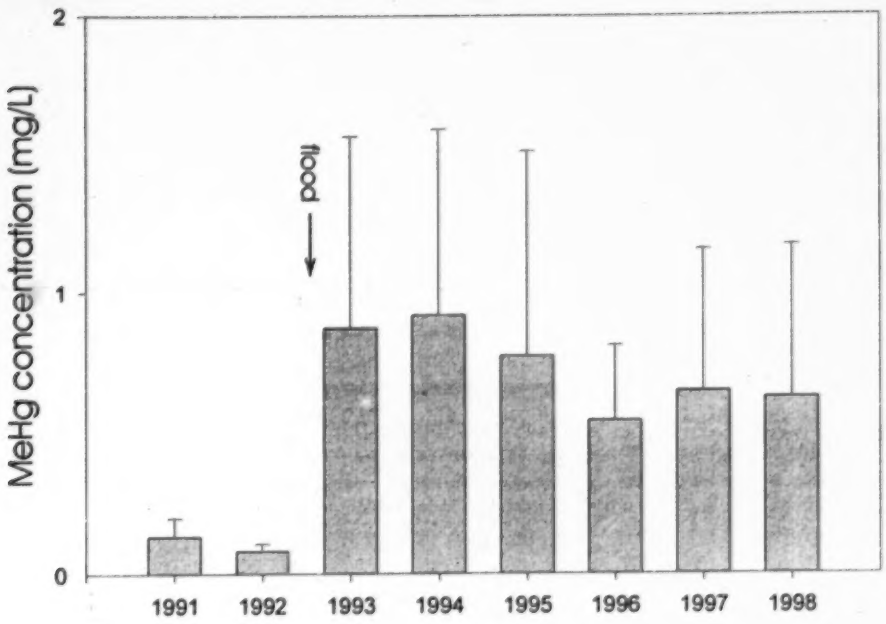

Figure 1. Mean (± std) annual MeHg concentration in the surface water of the central pond of the experimental reservoir. The reservoir was flooded in June of 1993.

reservoir. We also determined the impact of flooding and MeHg bioaccumulation on the reproductive success of these birds.

Tree swallows are widely distributed passerine birds that feed on aquatic insects emerging from near their nest site (St. Louis *et al.*, 1990). They nest readily in man-made nestboxes and, during their breeding cycle, feed primarily on emergent Dipterans (primarily chironomids) with no preference for particular species (Quinney and Ankney, 1985). Tree swallows are readily affected by both prey abundance and metal accumulation and make an ideal organism for food chain bioaccumulation studies (Kraus, 1989; Wiggins, 1990a).

METHODS

Site Description and the ELARP Experiment

A 16.7 ha riverine wetland with a 14.3 ha peatland surrounding a 2.4 ha pond (Lake 979; L979; Fig. 2) was experimentally flooded, providing the worst case scenario for organic carbon decomposition and Hg methylation (Kelly *et al.*, 1997). After two years of studying the natural biogeochemical cycling of MeHg and inorganic Hg in the wetland, the outflow was dammed and the water level was raised 1.3 m with water flowing in from an upstream lake (Lake 240). The flooding of the peatland increased the pond's surface area by 300% and the water volume by 600%. The effects of flooding on whole-ecosystem and process-level Hg cycling were quantified by comparing preflood and postflood results (Kelly *et al.*, 1997). Two oligotrophic lakes (Lakes 224 and 239; (Armstrong and Uthe 1971) were used as reference sites for this study to determine natural levels and interannual variation in Hg bioaccumulation in birds.

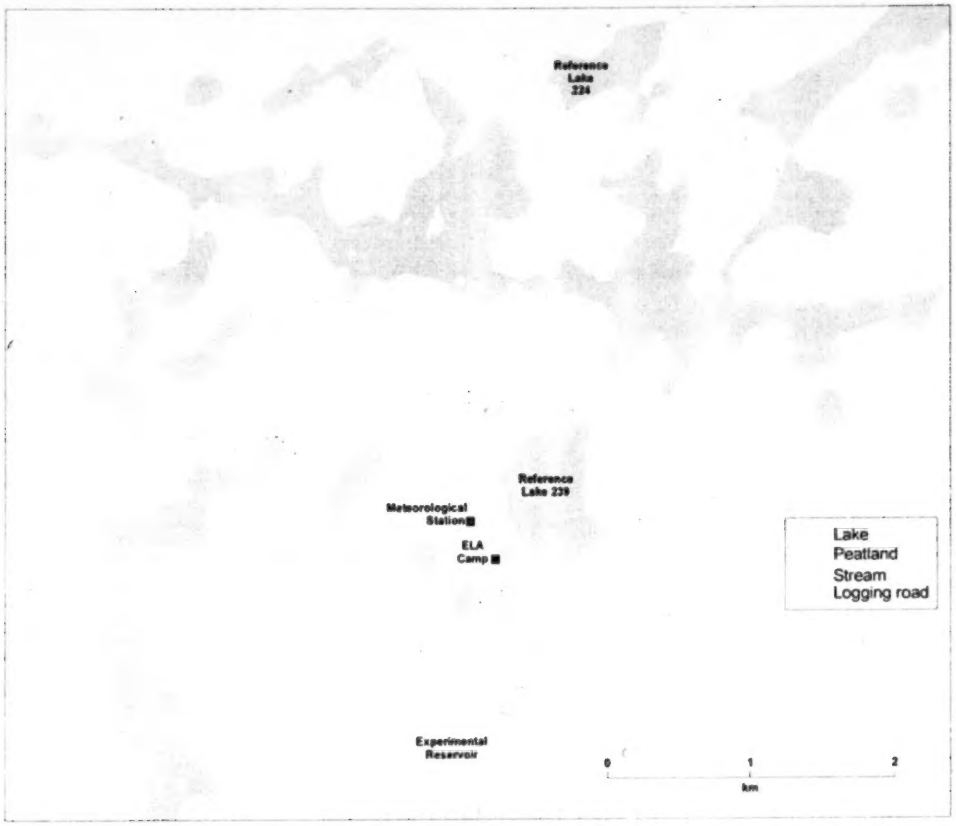

Figure 2. Relative location of lakes used in this study.

Dipteran Emergence

Tree swallows are aerial insectivorous birds that feed primarily on emergent dipterans from their nest site lakes (St. Louis, 1993a). Modified LaSage Harrison emergence traps (Rosenberg *et al.*, 1988) were used to quantify the biomass of dipteran emergence from the experimental site and to collect emergence for MeHg analyses. Ten traps with sampling jars containing 70% ethylene glycol antifreeze were deployed in a transect over the pond and peatland and sampled weekly during the ice-free season. Three additional emergence traps without ethylene glycol sampling jars were deployed over both the pond and peatland and were sampled three times each week for MeHg analyses using a modified Dust-Buster™ vacuum system (Marshall, 1982). Emergence sampled for MeHg analyses was stored in sterile Whirl-paks™ and frozen immediately after collection. Dipteran specimens were separated from other orders and freeze-dried prior to analyses. Dipteran tissues from 1992-1995 were analyzed for organic Hg (primarily MeHg) using a 3:2 methylene chloride:hexane extraction and cold-vapor atomic absorption spectrophotometry as described by Armstrong and Uthe (1971) and Malley *et al.* (1996). A number of subsamples were also analysed by Flett Research Ltd. for MeHg (Winnipeg, Manitoba) using distillation, ethylation and atomic fluorescence (Horvat *et al.*, 1993) to confirm that the organic Hg extracted using methylene chloride:hexane was MeHg. Flett Research Ltd. analysed all Dipteran tissues from 1998. One or two replicate analyses, blanks, and spikes (32 ng) were done with every distillation set of eight samples. Duplicate samples were repeated if they deviated more than 15% from the mean. Spike recovery for emergence samples was 96%. Emergence on L979 was collected initially during the first four years of the experiment (1992-1995) and then again in 1998 as part of an alternate year monitoring program. No emergence was

collected or monitored for biomass flux on reference lakes used to monitor tree swallows. The full study of emergence biomass and MeHg flux from the entire catchment and reference wetland during the 1991-95 portion of the ELARP was completed by A.P. Weins and D.M. Rosenberg (Freshwater Institute, unpublished data).

MeHg Bioaccumulation

We began monitoring tree swallows nesting at the experimental reservoir and reference lakes (lakes 224 and 239; Fig. 2) in 1992 (Fig. 3). All birds were handled in accordance with recommendations of the Canadian Council on Animal Care (Protocol #240804, University of Alberta). Each spring, prior to lake ice-out and swallow arrival, nestboxes were installed, and mounted on either wooden posts about 1 meter from shore, or snags or live trees along the shoreline. On reference lakes 239 and 224 we installed five and seven nestboxes, respectively. Average occupancy was 43% of nestboxes on lake 224 and 11% on lake 239. On the experimental reservoir we installed six nestboxes prior to flooding (1992 and 1993) and 11 nestboxes after flooding (1994-1999). On average, 74% of nestboxes at the experimental reservoir were occupied over the course of the study.

Because tree swallows feed primarily on insects emerging from near their nestsite, MeHg accumulation in nestlings is a reflection of bioavailable MeHg in the waterbody on which they were raised. Conversely, MeHg concentrations in adult tree swallows may reflect environmental levels of Hg in the wintering grounds and along migration routes. Although we did not actively sample adult tree swallows, we did occasionally find dead adults in nestboxes during the early spring and analyzed them for

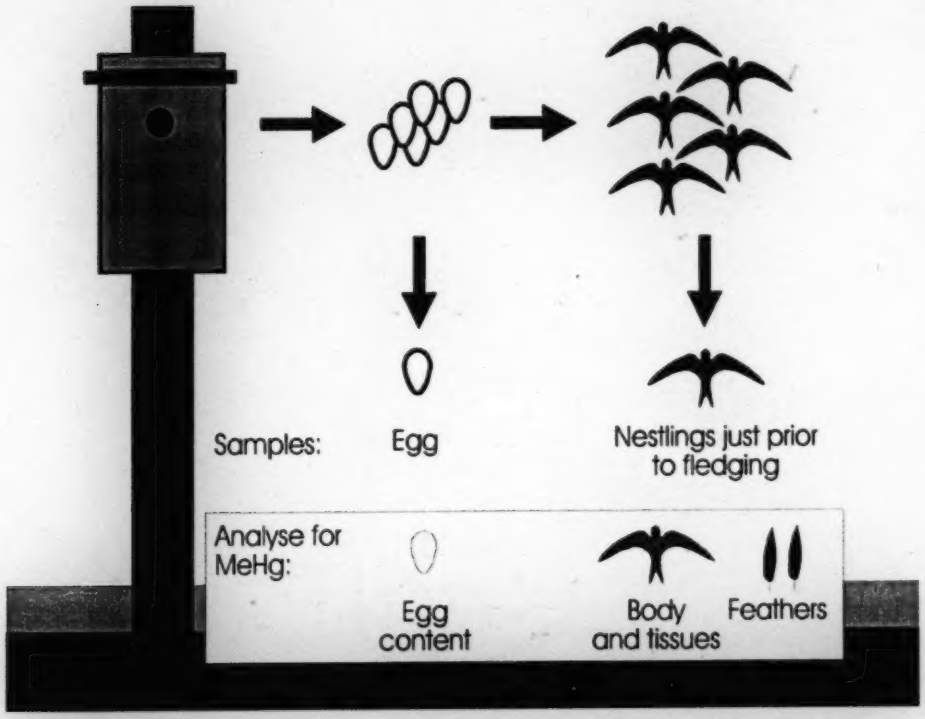

Figure 3. Sampling protocol to quantify MeHg bioaccumulation in embryo and nestling tree swallows.

MeHg concentrations and burdens. These adults were collected soon after death and treated in the same manner as nestlings collected later on in the season.

We used the following protocol to quantify MeHg bioaccumulation in swallows nesting near the various sites (Fig. 3). To quantify the transfer of MeHg from the mother to her young, one egg was collected from each nestbox prior to hatching (Environment Canada migratory bird scientific/capture permit #CA-0016). To determine the amount of MeHg accumulated through ingestion of emergent insects during nestling growth, two or three nestlings were collected prior to fledging (approximately 17 days old). Both eggs and fledglings were frozen, usually within one hour of collection. All tissues were stored in Ziploc™ bags or sterile Whirl-paks™ and freeze-dried prior to analysis. Other studies in our lab have shown that freeze-drying tissues resulted in no loss of MeHg (Paterson *et al.*, 1998). Eggshells were removed from frozen embryos, and whole embryos were analyzed. Feathers were removed from nestlings and analyzed separately because contaminants are often shunted into feathers, where they can be bound and excreted annually during molt. Feathers were first washed using warm deionized water, then homogenized using cleaned stainless steel scissors. Stomachs and intestines were removed from nestlings using acid-washed (dilute 10% HCl) stainless steel scalpels, and contents were rinsed out with deionized water prior to tissue analyses. Whole nestlings were ground using a stainless steel coffee grinder that was rinsed between uses with dilute HCl (Hall *et al.*, 1998). Beginning in 1996, we analyzed three target tissues (brain, liver and breast muscle) for MeHg bioaccumulation. These tissues were dissected out of each nestling using dilute acid-washed stainless steel scalpels and tweezers. All target tissues and embryos were homogenized using an acid-washed glass mortar and pestle. Brain tissues from birds in the same nestbox were pooled due to small brain biomass.

Tissues were handled exclusively in Hg clean rooms at the Freshwater Institute, Winnipeg, MB and the Experimental Lakes Area field station.

A random sample of feathers, whole birds, and isolated target tissues were analyzed for both THg and MeHg. MeHg was analyzed by Flett Research Ltd. as previously described for dipteran analyses. Results from these analyses confirmed that almost all Hg in tree swallow tissues is MeHg (see results). Therefore, we used the cheaper THg analytical technique to quantify MeHg accumulation in bird tissue.

THg was analyzed at the Freshwater Institute using cold vapor atomic absorption spectrophotometry (AAS) (Armstrong and Uthe, 1971; Hendzel and Jamison, 1976). Certified Mercury Reference Solution for Atomic Absorption was used for standardization (0.5, 1, 5, 10, 25, 50, 100, 200 ng THg; r^2 = 0.99-1). Two method blanks and one to three NCR Certified Reference materials (NRCC dogfish muscle: Dorm-1, dogfish liver: Dolt 2, and lobster hepatopancreas: Tort-2) were analyzed using the same procedures as the samples. Recovery for all reference material was 92.3%. Spikes of 500 ng and 1000 ng were added to some feather tissues, with a mean (± std) spike recovery of 97.2 ± 10.5%. Mean (± std) coefficient of variation (CV) between replicates was 8.73 ± 7.85%. Interyear calibrations were conducted between periods of analyses and showed variability similar to that between replicates (CV = 11.73%).

We calculated Hg burdens for individual tissues from the dry weight of the tissue and its concentration of MeHg. Total burden for a given nestling was simply the sum of all tissue burdens (feather, body, muscle, liver, brain). Similarly, whole nestling concentrations were calculated by dividing total nestling Hg burden by total dry weight of all combined tissues.

Because parents tend to feed similar food items to all nestlings within a clutch, we calculated mean Hg concentrations and burdens for all birds within the same clutch prior to calculating mean site values. We also pooled data from both reference lakes prior to calculating mean Hg concentrations and burdens to increase sample sizes. Any late nesting attempts (e.g., due to failed first clutches) were excluded from all analyses because as lake temperatures increase so do Hg methylation and MeHg bioaccumulation in dipteran emergence.

We predicted that there would be elevated MeHg concentrations in all organisms throughout the food chain following the large increase in the MeHg concentration in the water of the experimental reservoir after flooding (Kelly *et al.*, 1997). To determine if flooding had an impact on MeHg accumulation in tree swallows, we used a one-way analysis of variance (ANOVA-SigmaStat 4) to compare nestling tissue concentrations and MeHg burdens between years. A pair-wise multiple comparisons Tukey test was used to determine which years were significantly different from the others. To identify natural interannual variation, we compared MeHg concentrations and burdens in tree swallows at reference lakes using the same procedure as for the experimental reservoir (one-way analysis of variance, and pair-wise multiple comparison Tukey test). We compared individual tissue concentrations from the experimental reservoir with those from reference lakes using non-parametric Mann-Whitney tests to compensate for non-normal distributions. To examine the relationship between feather and body tissues we used linear regression to correlate feather with muscle, liver and brain concentrations. Conversion ratios of feather:liver:muscle were then calculated for all birds by dividing feather and liver concentrations by muscle concentrations.

This experimental design is an impact analysis (Eberhardt and Thomas, 1991; Underwood, 1992) and does not replicate experimental units, due to the difficulty of replicating whole-ecosystem experiments. We were further limited statistically on the reference lakes by the small number of clutches laid and the lack of successful clutches during the first year of the experiment (1992), leaving us with only one comparable pre-impact year for reference lakes. However, the data are strong enough to show clear trends and we use inferential statistics to emphasize our results, acknowledging the implication of pseudoreplication as described by Hurlbert (Hurlbert, 1984).

Reproductive Success

In conjunction with monitoring MeHg bioaccumulation in tree swallows, a number of reproductive parameters were measured to determine whether flooding and increased levels of MeHg had an effect on reproductive success. Clutch initiation date, number of eggs per clutch, egg size, incubation length, hatching success, growth rates of nestlings, and fledging success were quantified for all clutches, as outlined by St. Louis and Barlow (St. Louis, 1993b). Growth rates were quantified by measuring wing, tail, tarsus, and bill lengths, bill width, and body weight every 2-3 days between hatching and fledging. Due to funding constraints no reproductive parameters were measured during 1996 and growth rates of nestlings were not quantified in 1997 and 1999. We also omitted all reproductive parameters measured for females less than two-year-old, identified by non-iridescent brown feathers on their back. Young females tend to be inexperienced breeders and their reproductive success may be lower than older birds (DeSteven, 1978, St. Louis and Barlow, 1993b).

To examine the impact of flooding on reproductive success, we used t-tests to compare the different reproductive parameters between preflood and postflood. We accepted *p*-values <0.01 as indicating significant differences, due to the large number of parameters tested (Rice, 1989). We pooled all reproductive parameters measured at the two reference lakes prior to conducting t-tests between the preflood (1993) and postflood (1994-98) years. We tested for differences in the growth rates between preflood and postflood years by comparing linear slopes of temporal changes of wing, tail, and bill lengths (r^2 =0 .92-1.0 for wing length; 0.91-1.0 for tail length; and 0.80-1.0 for bill length). Sigmoidal changes in bill width, tarsus length, and weight were fitted to a logistic model (r^2= 0.87-1.0 for bill width; r^2 = 0.90-1.0 for tarsus length; r^2 = 0.91-1.0 for weight) and used to compared the growth rate constant (K) and the asymptote (a) or upper limit of growth (Ricklefs, 1967; Zach and Mayoh, 1982). For bill width we included days 1 to 13 and for weight, days 1 to 15, thus avoiding the recession period in both measurements. After removing the females less than two years old from our reference lake data set there were only two clutches for the preflood period. Thus we only compared growth rates and constants from postflood reference lakes with those from the postflood experimental reservoir. We used non-parametric Mann-Whitney tests for all comparisons, to account for non-normal distributions or inequalities in variance of some growth rates or constants.

RESULTS

Dipteran Emergence

The mean (± std) monthly (May-October) biomass of dipteran emergence (primarily Chironomidae) from the pond of the experimental reservoir was greater in postflood

years (197 ± 238 ng/m^2/month) than in preflood years (73.9 ± 40 ng/m^2/month; Fig. 4a) although there was large variation between months (A.P. Weins and D.M. Rosenberg, unpublished data). Similarly mean (± std) postflood MeHg concentrations in emergent dipterans were greater (111 ± 2.4 ng/g dry weight) than preflood (43.8 ± 15.8 ng/g dry weight) (A.P. Weins and D.M. Rosenberg, unpublished data). Six years after the initial flooding of the reservoir dipteran MeHg concentrations were still elevated (119 ± 21.6 ng/g dry weight; Fig. 4b). Because postflood biomass of emergence and MeHg concentrations in Diptera were greater than preflood, postflood flux of MeHg out of the reservoir via dipteran emergence was also greater. These results only include dipteran emergence directly over the pond of the reservoir and not over the flooded peatland.

The Chemical Form of Hg in Tree Swallows

We found a near 1:1 relationship between concentrations of THg and concentrations of MeHg in all tissues analyzed from tree swallows ($r^2 = 0.88$, slope = 0.91, $p < 0.001$; Fig. 5), excluding two liver samples with much lower concentrations of MeHg than concentrations of inorganic Hg. From the 20 liver samples that we analyzed for both MeHg and THg all but those two fit a 1:1 relationship ($r^2 = 0.83$, slope = 1.06, $p < 0.001$). Although these two liver samples suggest that there might be MeHg demethylation occurring in some proportion of livers, due to funding constraints we could not directly analyze more liver samples for MeHg.

Hg in Adult Tree Swallows

We found nine adult tree swallows dead in nestboxes over the course of the study. Hg concentrations and burdens in adults were highly variable. Body concentrations ranged

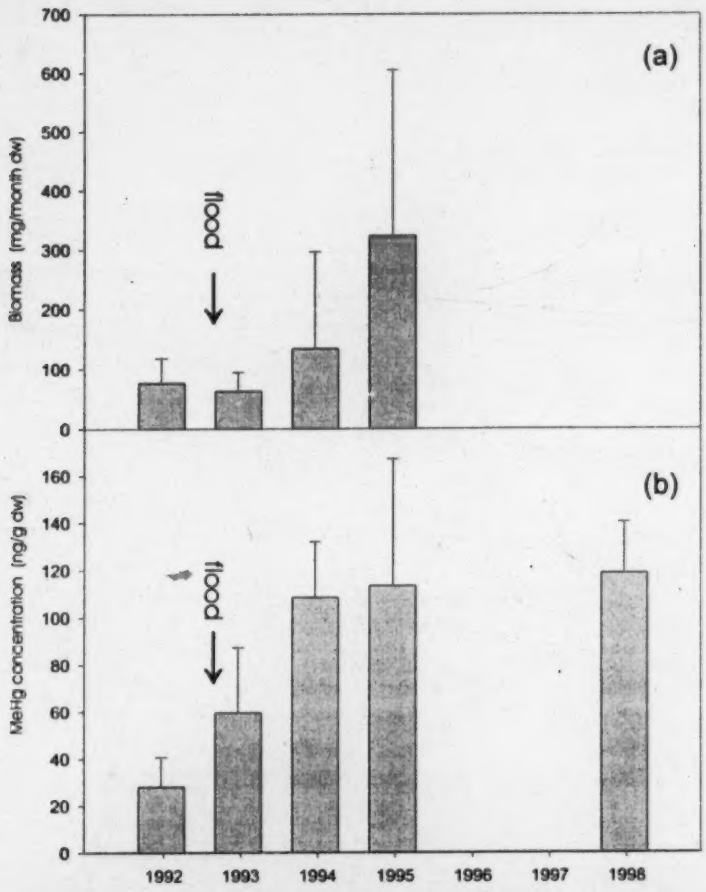

Figure 4. Mean monthly biomass of dipteran emergence from the pond of the experimental reservoir before and after flooding (a), and mean monthly MeHg concentration in emergence (b).

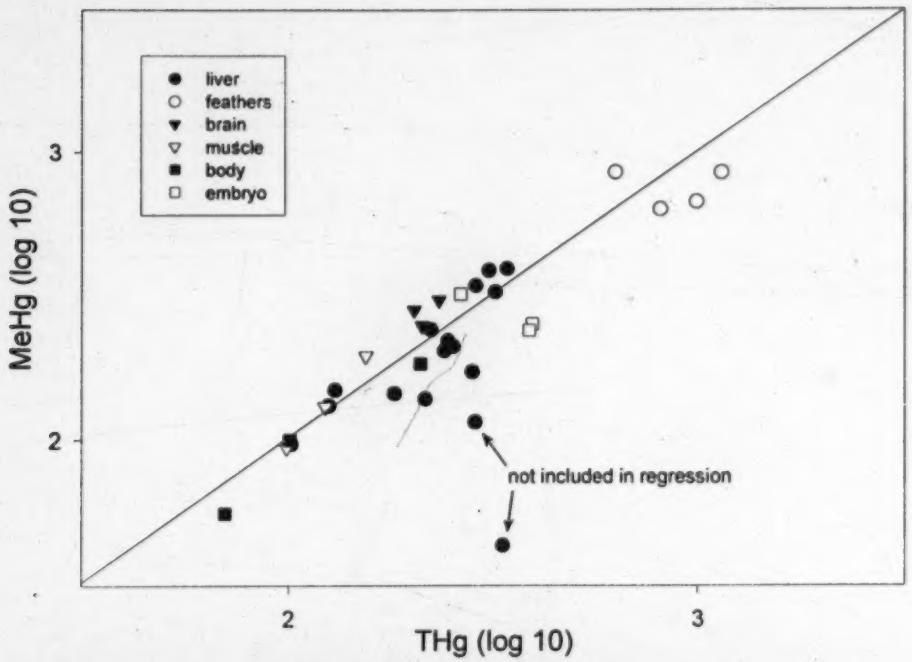

Figure 5. Log-log relationship between MeHg and THg concentrations in nestling tree swallow tissues. Indicated liver samples were not included in the regression because Hg demethylation may have occured in these livers (see discussion).

from 214-1110 ng/g dry weight (mean = 517 ng/g dry weight; coefficient of variation = 57%). Feather concentrations ranged from 843-2740 ng/g dry weight (mean = 1690 ng/g dry weight; coefficient of variation = 41%). Total body burden of Hg in adult tree swallow ranged from 2760-8540 ng (mean of = 4760 ng). Although we did not include adult bird tissue in our analyses of MeHg:THg, we assume that the majority of the Hg in adult birds is methylated.

Hg Bioaccumulation in Tree Swallow Embryos and Nestlings

Concentrations:

MeHg concentrations in embryos were variable between years at all sites (Table 1). Mean (± std) MeHg concentrations at the experimental reservoir and references lakes were 365 ± 35 ng/g and 270.0 ± 18 ng/g, respectively (Table 1). Concentrations of MeHg in embryos collected in 1992, a preflood year, were higher than other years ($p < 0.01$) on the experimental reservoir, whereas there were no differences between years in concentrations of MeHg in embryos at reference lakes ($p = 0.31$) (Table 1).

Mean concentrations of MeHg in nestling bodies were 81.6 ± 5.1 ng/g at the reference lakes and 82.8 ± 2.2 ng/g at the experimental reservoir in the two years prior to flooding (Table 1). Mean concentrations of MeHg in nestlings bodies were 130 ± 11 ng/g after flooding (Table 1). Although mean concentrations of MeHg in postflood nestling bodies were 36.3 % higher than concentrations measured preflood, only nestlings collected in 1994 were significantly higher than all other years ($p < 0.001$).

Postflood feather MeHg concentrations were also higher than preflood (Table 1). Preflood mean concentration of MeHg in feathers was 747 ± 43 ng/g at reference lake

Table 1. MeHg concentration in tree swallow embryos and nestling bodies and feathers from the experimental reservoir and reference lakes.

		Embryo			Body			Feather		
		Concentration (ng/g)	SE	N	Concentration (ng/g)	SE	N	Concentration (ng/g)	SE	N
Experimental Reservoir										
Preflood	1992	518	25.9	4	80.7	3.2	4	947	34.2	4
	1993	476	50.0	5	86.0	7.0	6	796	26.6	6
Postflood	1994	364	47.1	6	110	4.7	6	1070	68.6	6
	1995	314	38.8	8	188	15.1	8	1090	63.8	8
	1996	285	17.4	9	124	10.6	9	1290	43.9	9
	1997	318	21.8	9	120	4.2	9	1330	61.5	9
	1998	413	41.8	6	117	4.9	7	1110	55.3	7
	1999	230	28.4	6	123	5.3	6	1340	75.4	6
Reference Lakes										
	1992	267	31.6	3						
	1993	366	59.2	4	70.4	7.5	4	604	75.0	4
	1994	276	42.1	3	95.6	9.9	4	924	81.8	4
	1995	288	8.2	3	70.2	5.1	3	648	46.7	3
	1996	307	7.1	2	75.0	12.8	2	735	46.0	2
	1997	220	7.1	2	102	32.2	2	851	174	2
	1998	214	70.5	3	81.7	6.3	3	677	43.3	3
	1999	221	12.0	2	67.5	5.0	3	788	21.8	3

and 872 ± 75 ng/g at the experimental reservoir. Postflood mean feather MeHg concentration was 1210 ± 53 ng/g in nestlings collected at the experimental reservoir, and was higher ($p = 0.01$) than during the preflood period, with the exception of 1992 versus 1994 and 1995. There were no differences in feather MeHg concentrations between years at reference lakes ($p = 0.06$).

Concentrations of MeHg in tissues dissected from nestlings from 1996-1999 were higher in those collected from the experimental reservoir than in those collected from the reference lakes (Fig. 6). For all nestlings combined, concentrations of MeHg were highest in feathers, 78% lower in liver, 84% lower in brain, and 90% lower in muscle tissue. Liver, muscle and brain concentrations correlated with feather concentrations ($r^2 = 0.59$, $p < 0.001$ for liver; $r^2 = 0.52$, $p < 0.001$ for muscle; $r^2 = 0.35$, $p < 0.001$ for brain; Fig. 7). We calculated a conversion ratio for feather:liver:muscle of 10:2:1 for birds from all sites. There was some variation in this ratio between individuals, ranging from 6.8-16.3 for feather:muscle, and from 1.5-2.6 for liver:muscle.

Body Burdens:

As with concentrations, embryo Hg burdens at all sites were variable between years (Table 2). Mean embryo burdens ranged from 44-111 ng MeHg. We found no difference between preflood and postflood years on the experimental reservoir ($p = 0.17$) or on reference lakes ($p = 0.58$). Embryo burdens, representing the transfer of Hg from mother to young, ranged from 1.2-10.7% of the eventual total Hg burden in pre-fledging nestlings (Table 2).

Mean postflood MeHg burdens in nestlings at the experimental reservoir were higher (2200 ± 102 ng; $p < 0.001$) than before flooding (1210 ± 150 ng; Fig 8).

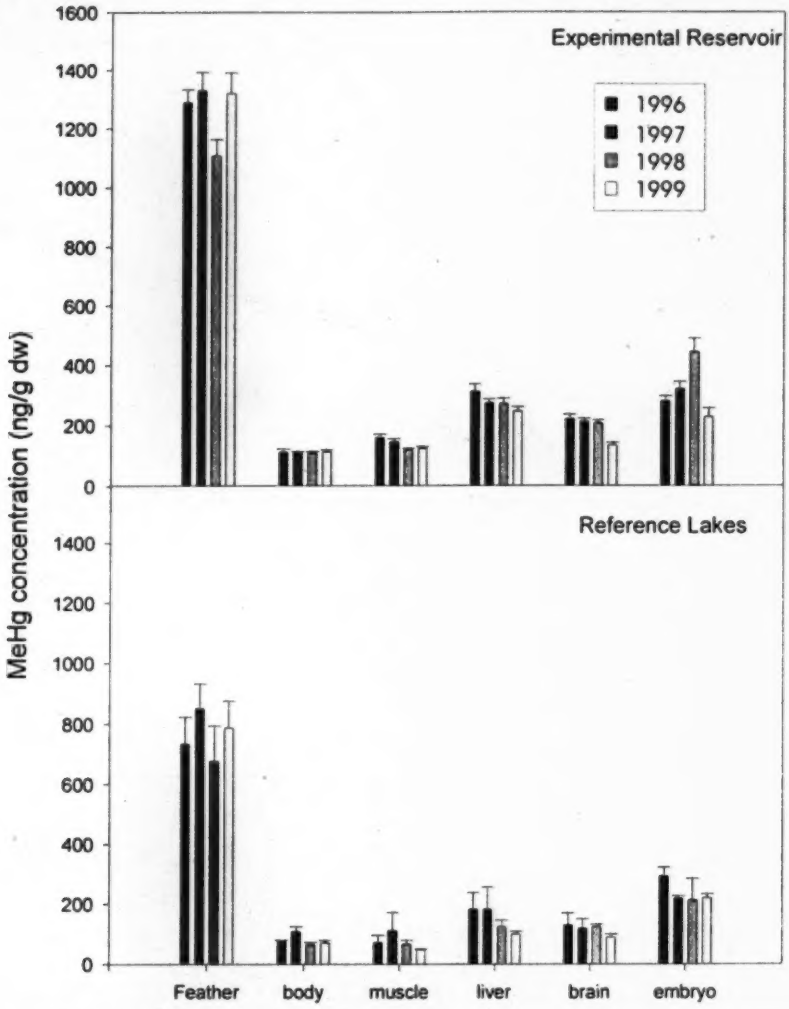

Figure 6. MeHg concentration in nestling tree swallow tissues near the experimental reservoir and reference lakes.

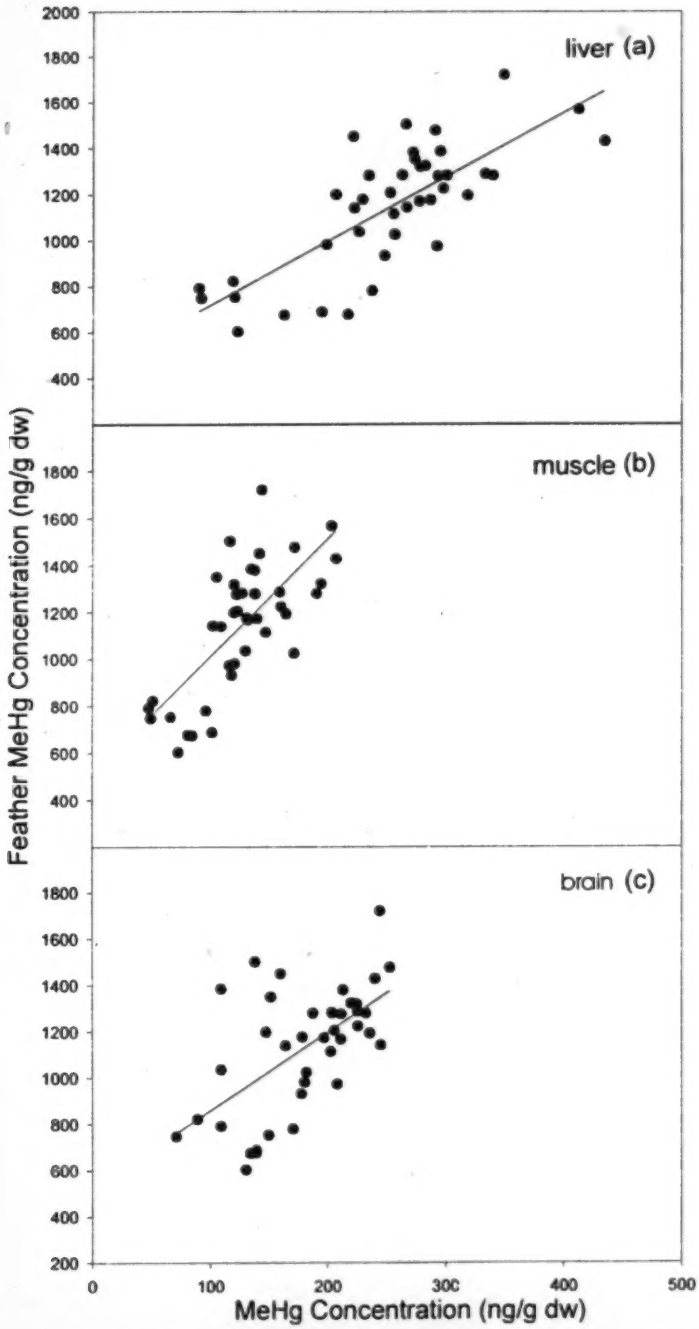

Figure 7. Plot of relationship between MeHg concentration in feathers and liver liver ($r^2 = 0.59$, $p < 0.001$) (a), muscle ($r^2 = 0.52$, $p < 0.001$) (b) and brain ($r^2 = 0.35$, $p < 0.001$) (c) in nestling tree swallows.

Table 2. MeHg burdens in tree swallow embryos and nestlings from the experimental reservoir and reference lakes.

		Embryo			Whole Bird			% Burden from embryo
		Burden (ng)	SE	N	Burden (ng)	SE	N	
Experimental Reservoir								
preflood	1992	108	5.5	4	1370	83.1	4	7.99
	1993	111	6.9	6	1060	121	6	10.7
postflood	1994	110	31.6	6	1930	109	6	5.94
	1995	81.4	9.5	8	1950	150	8	4.16
	1996	82.4	4.6	9	2410	105	9	3.46
	1997	86.7	12.8	4	2430	130.6	9	3.3.7
	1998	96.4	8.3	6	2080	88.6	7	4.69
	1999	61.5	5.7	5	2460	118	6	2.43
Reference Lakes								
	1992	67.62	10.1	3				
	1993	82.18	9.4	4	1050	168	4	8.21
	1994	55.61	24.1	3	1640	105	4	3.64
	1995	69.70	5.2	3	1150	60.3	3	6.12
	1996	77.98	20	2	1280	207	2	5.99
	1997	66.09	9.3	2	1460	383	2	4.66
	1998	43.96	15.8	3	1320	70.2	3	3.22
	1999	59.03	4.4	2	1400	83.5	3	4.03

Conversely, there was no difference in mean Hg burdens at the reference lakes between years ($p = 0.317$) although there was some year-to-year variation (Fig. 8).

Reproductive Success

Tree swallows on the experimental reservoir began nesting earlier in the postflood years than preflood. Mean clutch initiation date changed from day 151 ± 1.2 preflood to day 145 ± 0.8 postflood in clutches on the experimental reservoir ($p = 0.006$; Table 3). Tree swallows on the reference lakes establish nests on day 150 ± 1.0 in all years ($p = 0.07$ for 1992/93 versus 1994-99 on the reference lakes; Table 3). Postflood egg width and volume were higher than preflood at the experimental reservoir. Egg width was 13.4 ± 0.07 mm^3 preflood and 13.8 ± 0.06 mm^3 postflood ($p = 0.004$). Egg volume was 1670 ± 19 mm^3 preflood and 1820 ± 18 mm^3 postflood ($p < 0.001$). There were no between-year differences in egg width or egg volume at the reference lakes ($p = 0.07$ for egg width and $p = 0.083$ for volume). There were also no differences between preflood and postflood clutches in egg weight or length, eggs per clutch, total volume in the clutch, incubation length, hatchability, growth period, or fledging success at the experimental reservoir. Similarly, there were no differences between years in any of these same tree swallow reproductive parameters from reference sites (Table 3).

Both wing and bill length growth rates were higher in tree swallows nesting at the experimental reservoir after flooding than prior to flooding (Fig.9). Wing length growth rates changed from 4.2 ± 0.1-5.0 ± 0.1 mm/day ($p = 0.001$) and bill length growth rates changed from 0.17 ± 0.01-0.22 ± 0.01 mm/day ($p = 0.005$; Table 4). There were no differences in preflood and postflood tail length growth rates or growth constants for

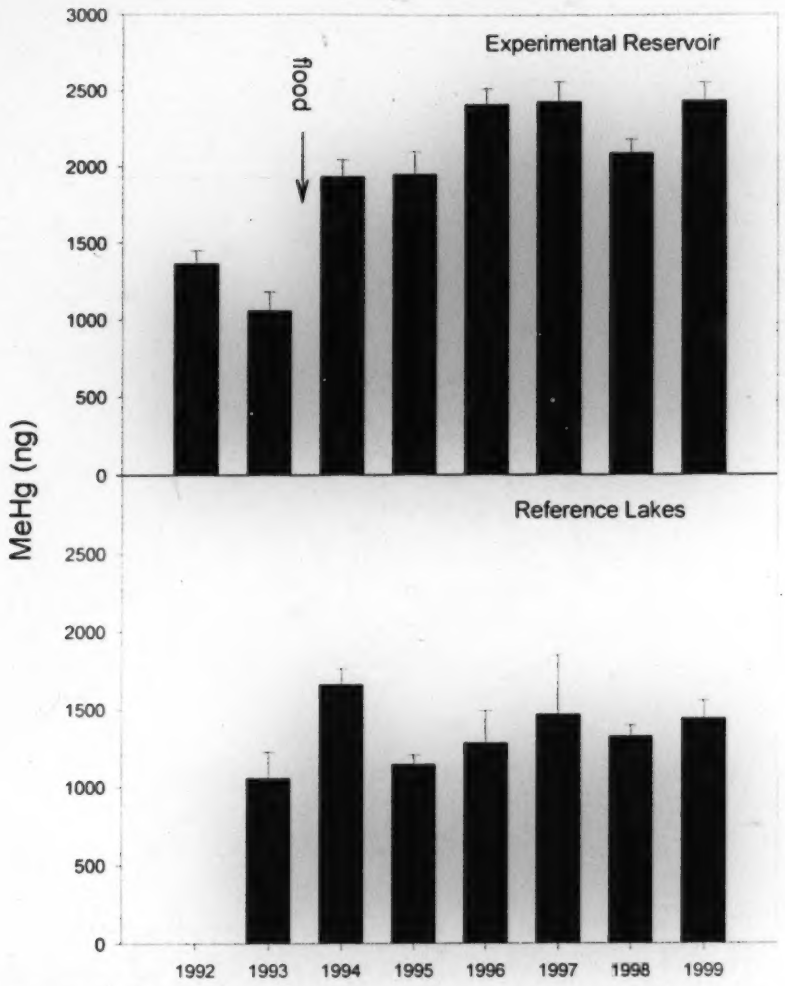

Figure 8. MeHg burdens in nestling tree swallows near the experimental reservoir and reference lakes.

Table 3. Mean values (±SE) for egg size and reproductive parameters examined in tree swallows nesting near the experimental reservoir and reference lakes. *P*-values are results of t-tests between preflood and postflood on the experimental reservoir (*), and preflood period and postflood period on the reference lakes (**).

	Preflood Experimental Reservoir			Postflood Experimental Reservoir			t-test*	92/93 Reference Lakes			94-99 Reference Lakes			t-test**
	mean	SE	N	mean	SE	N	*p*	mean	SE	N	mean	SE	N	*p*
Reproduction														
Initiation date (day of year)	151	1.2	8	145.2	0.8	41	0.006	153.8	1.2	5	150	1.0	18	0.07
No. of eggs/clutch	6.3	0.3	8	6.3	0.1	41	0.81	6.0	0.3	5	5.7	0.3	20	0.3
Incubation length (days)	19.3	0.5	8	19.9	0.3	34	0.36	19.5	0.5	2	19.6	0.5	14	0.95
Hatchability (%)	87.8	6.1	8	86.2	4.4	41	0.88	53.3	22.6	5	73.0	7.9	19	0.3
Growth period (days)	17.0	0.7	6	15.4	0.2	27	0.012	17.5	0.5	2	15.8	0.4	13	0.1
Fledging success (%)	75.0	16.4	8	89.3	5.1	29	0.27	66.7	33.3	3	98.6	1.4	14	0.04
Egg Size														
Weight (g)	1.7	0.03	8	2.0	0.04	41	0.039	1.7	0.06	5	1.6	0.06	19	0.6
Length (mm)	18.5	0.19	8	19.0	0.1	41	0.059	18.7	0.5	5	19.0	0.2	19	0.6
Width (mm)	13.4	0.07	8	13.8	0.06	41	0.004	13.2	0.1	5	13.6	0.09	19	0.07
Volume (mm^3)	1670	19.3	8	1820	17.5	41	<.001	1660	41.7	5	1770	30.4	19	0.08
Total volume in clutch (mm^3)	10480	505	8	11520	245	41	0.084	9900	362	5	10020	536	19	0.9

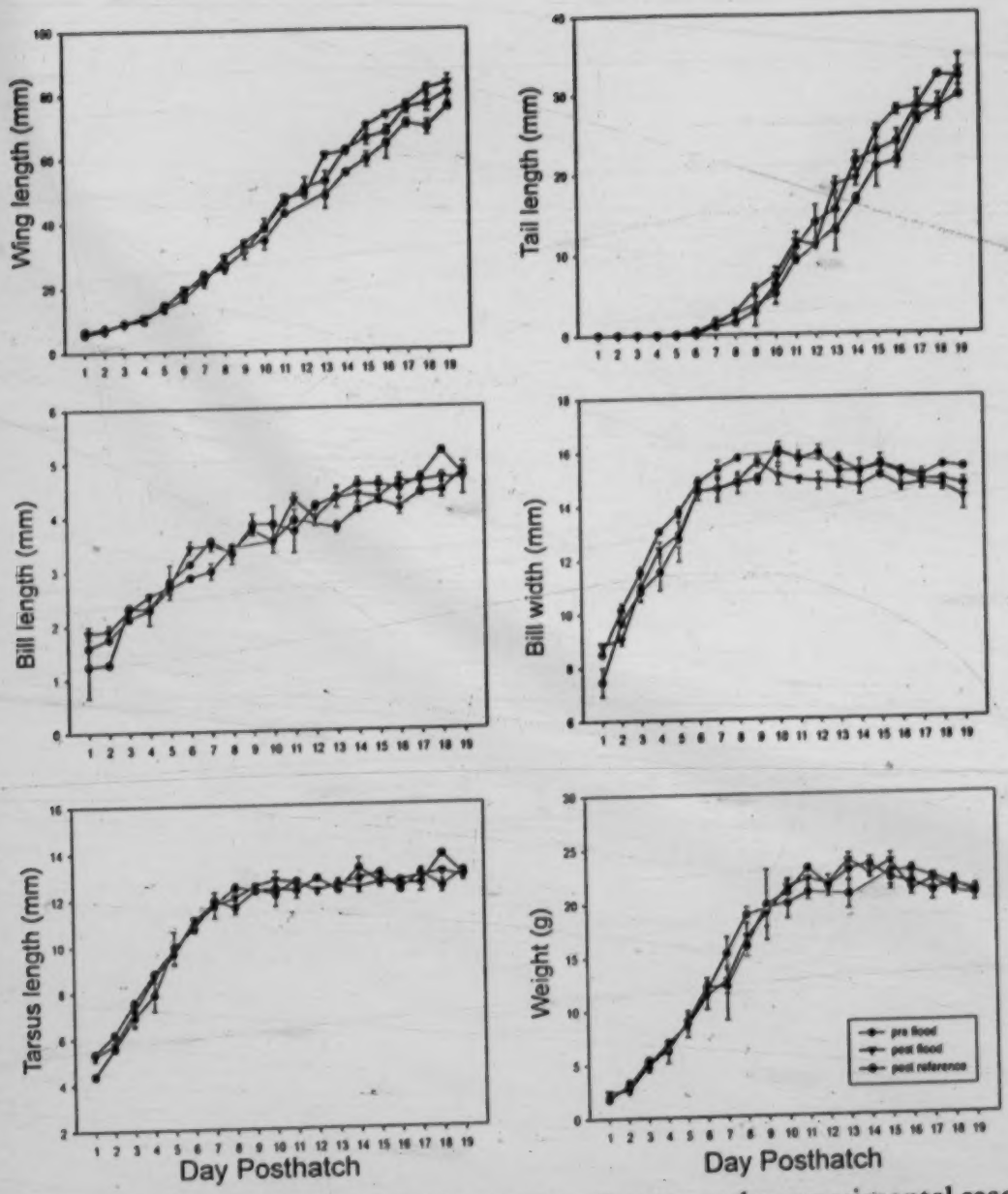

Figure 9. Growth plots for nestling tree swallows near the experimental reservoir (preflood and postflood) and during the postflood period near the reference lakes.

Table 4. Growth rates and growth rate constants (K , the logistic equation) for growth measurements on nestling tree swallows from the experimental reservoir and reference lakes. *P*-values are results of Mann-Whitney tests between preflood and postflood periods on the experimental reservoir (*), and postflood on the experimental reservoir and postflood period on the reference lakes (**).

	Preflood Experimental Reservoir			Postflood Experimental Reservoir			Mann-Whitney*	1994-1998 Reference Lakes			Mann-Whitney**
	Mean	SE	N	Mean	SE	N	*p*	Mean	SE	N	*p*
Growth Rate											
Wing Length	4.2	0.095	6	4.96	0.06	20	0.001	4.82	0.14	13	0.95
Tail Length	2.65	0.084	6	2.89	0.07	20	0.055	3.01	0.08	13	0.17
Bill Length	0.17	0.007	6	0.22	0.01	20	0.005	0.203	0.01	12	0.69
Growth Constant											
Bill Width	3.68	0.2	6	4.92	0.19	19	0.12	5.34	0.45	10	0.08
Tarsus Length	4.53	0.34	6	4.44	0.16	21	0.98	4.71	0.29	12	0.83
Weight	6.16	0.43	8	6.86	0.3	19	0.58	6.92	0.28	12	0.91

bill width, tarsus length, and weight (Table 4). Similarly, preflood and postflood asymptotes for bill width, tarsus length, and weight were not different.

We found no significant difference between any of the growth characters measured (growth rate, growth constant, and asymptote) in postflood nestlings from the experimental reservoir and nestlings from reference lakes during the same period (1994-1998), including wing and bill length. Unfortunately, mature females (> two years old) on reference lakes raised only two successful clutches during the preflood period (both in 1993). Given these low numbers, we could not statistically compare preflood and postflood periods on reference lakes.

DISCUSSION

We found that tree swallows nestlings from around the experimentally created reservoir had higher MeHg tissue concentrations and body burdens after flooding than prior to flooding. MeHg burdens were elevated in nestling tree swallows during the first year of flooding and remained high during six consecutive breeding seasons. Conversely, MeHg burdens in nestling tree swallows on reference lakes in the area did not increase during the course of the study. A change in MeHg concentrations and body burdens in tree swallows nestlings on the experimental reservoir mirrored changes in concentrations observed in both the water of the experimental reservoir and in dipteran insects emerging from the reservoir.

Dipteran Emergence

Preflood mean MeHg concentrations in emergent dipterans were lower than those of the first three postflood years and concentrations were still elevated in 1998, six years after initial flooding. Hg methylation primarily occurred in the sediments and flooded vegetation and peat of the experimental reservoir (Kelly *et al.*, 1997) where larval Diptera lived prior to emergence. Parkman and Markus (Parkman and Meili, 1993) found that Hg uptake by sediment-dwelling organisms was likely the result of elevated Hg concentrations in both their diet and physical surroundings. Postflood mean monthly flux of MeHg in dipteran emergence from the central pond was higher because of both elevated MeHg concentrations in postflood insects and elevated biomass of emergents from the reservoir. Given that tree swallows at the ELA are known to feed on emergents from their nestsite lake, we conclude that the experimental reservoir provided a large source of MeHg to tree swallows nesting near it, especially when emergence biomass is high (St. Louis *et al.*, 1990). Similarly, an examination of bioaccumulation of heavy metals (not including Hg) in nestling tree swallows revealed that metal body burdens in adult midges were accumulated from contaminated sediments and that ingestion of these insects resulted in accumulation of metals in nestling tree swallows(Kraus, 1989). Bishop *et al.* (1995) also showed that Hg concentrations in tissues of tree swallows and red-winged blackbirds (*Agelaius phoenicues*) correlated with Hg levels found in sediment from the lakes near their breeding sites.

Postflood MeHg concentrations in Diptera (62.7-202.4 ng/g dry weight) were below those known to cause toxicological effects in birds, but near those that may cause reproductive or behavioural impairment. For example, in a controlled feeding experiment of three generations of mallard ducks, females laid fewer eggs and produced

fewer ducklings, and ducklings were less responsive to warning calls and hypersensitive to fright stimuli after being fed 500 ng/g dry weight MeHg (Heinz, 1979). Similarly Barr (1986) found reductions in egg laying and territorial fidelity in common loons (*Gavia immer*) consuming prey with 300-400 ng/g wet weight MeHg.

The Chemical Form of Hg in Nestling Tree Swallows

We found a strong 1:1 relationship between MeHg and THg in all tree swallow tissues, confirming that most Hg in nestling tree swallows is MeHg. Studies have demonstrated that all mercury in feathers is MeHg and it has a high affinity for sulf-hydryl groups found in the amino acids of feather keratin (Burger, 1997; Thompson and Furness, 1989). Thompson and Furness (1989) also found that all Hg found in muscle of a number of species of seabirds was MeHg, although others have found less than 100% MeHg in muscle tissue (Kim *et al.*, 1996). The relationship of MeHg:inorganic Hg found in liver is less clearly understood and varies considerably between studies. Thompson and Furness (1989) found that seabirds with low Hg burdens have close to 100% hepatic MeHg. However, some large, long-lived, slow-molting species (most commonly from the order Procellariiformes) have more than 90% inorganic mercury in their livers despite high MeHg concentrations in other tissues. As a result, some researchers have concluded that demethylation occurs in the livers of these species (Kim *et al.*, 1996; Lock *et al.*, 1992; Thompson and Furness, 1989, Thompson *et al.*, 1990). We found 100% hepatic MeHg in most of the tree swallows we analyzed. However, there were two individuals with considerably lower hepatic MeHg:THg. It is possible that Hg demethylation may occur in tree swallow livers; however, additional examinations of

both the demethylation process and proportion of MeHg:THg in specific species of birds are required.

Hg in Adults Tree Swallows

Hg burdens in birds should depend on levels of Hg in the food they consume, their length of exposure to Hg (i.e., their age), and/or stage of molt because birds shed Hg in their feathers (Scheuhammer, 1987). Tree swallows breeding at our study site winter in the southern United States, Central America, and northern South America (Robbins *et al.*, 1983) and undergo one complete molt annually between mid-July and early November (Stutchbury and Rohwer, 1990). All adult tree swallows we collected were found in early spring, following migration and exposure on their wintering grounds, and most likely varied in age. In the limited number of adult tree swallows that we analyzed, Hg concentrations were highly variable. This is to be expected in birds of the same species that are possibly from different wintering areas and of different age, and is common in all birds. Hg concentrations ranged from 80-330 ng/g THg in muscle of adult mallard (*Anas platyrhnchos*) and black ducks (*Anas rubripes*) surveyed nationwide in the United States (Finley and Stendell, 1978). Similarly THg concentrations in adult common loon blood from five regions across North America ranged from 600-3600 ng/g (Evers *et al.*, 1998).

Hg in Tree Swallow Embryos

Embryonic MeHg burdens represent the transfer of MeHg from mother to young. As with adults arriving at our breeding sites, we found highly variable MeHg concentrations in tree swallow embryos. There were no between-year differences between pre- and

postflood embryonic MeHg concentrations or burdens, although embryonic MeHg concentrations and burdens from the experimental reservoir were in general higher than those from the reference lakes (Tables 1 and 2). It has been suggested that embryonic Hg concentrations reflect the mother's recent dietary uptake more than her accumulated tissue stores (Wolfe, 1998). Higher embryonic MeHg concentrations and burdens on the experimental reservoir, relative to reference lakes, are most likely because wetlands tend to be sites of high natural Hg methylation rates (St. Louis *et al.*, 1996; Winfrey and Rudd 1990).

The lack of difference between preflood and postflood embryonic MeHg concentrations and burdens is likely because the reservoir was drawn down each fall and the existing wetland was not reflooded until late May of the next year, when tree swallows had already begun to lay their eggs. Hg methylation rates in the catchment rose significantly only after flooding each spring (Kelly *et al.*, 1997). DesGranges *et al.* (1998) also found no difference between egg Hg concentrations in ospreys (*Pandion haliaetus*) nesting near reservoirs and those near natural lakes. Because large reservoirs thaw later than most natural lakes and rivers, DesGranges *et al.* (1998) suggested that all ospreys fed in natural environments until reservoirs opened up later in the spring. According to Becker (Becker, 1992), who examined Hg levels in clutch sequence of Charadriiformes (Plovers, Sandpipers, Gulls, Terns, and Jaegers), the first egg laid contained up to 39% more Hg than the second or third eggs laid. Because we do not know the order in which eggs were laid, or which egg was sampled within a clutch, variation in Hg loading between eggs may explain some of the variability in embryonic MeHg concentrations and burdens.

We chose to analyze freeze-dried tissues to eliminate variability associated with partial desiccation of wet samples during freezing and storage. By drying samples we reduced the total nestling weight by between 60 and 75% (but considerably less for feathers) and as a result, concentrated the Hg in tissues. Many of the studies we have used for comparison purposes used wet weight Hg concentrations that would be much higher if given on a dry weight basis.

In a controlled feeding experiment with ring-necked pheasants (*Pheucticus ludovicianus*), eggs containing 500-1500 ng/g wet weight showed lower hatchability (Fimreite *et al.*, 1971). Heinz (1979) also found a decline in reproductive success in mallard ducks with egg Hg concentrations of 860 ng/g wet weight. Our mean embryo concentrations ranged from 213-518 ng/g dry weight and, with the exception of one that was 710 ng/g dry weight, all individual embryo concentrations were below 600 ng Hg/g dry weight. Again, if we account for the fact that our concentrations were measured on a dry weight basis, all our embryo were well below the suggested threshold for overt toxic effects.

Hg in Nestling Body and Tissues

Preflood MeHg burdens were higher than postflood MeHg burdens in tree swallow nestlings from around the experimental reservoir. In the first two years (1994 and 1995), postflood MeHg burdens increased by a factor of 1.6 over preflood burdens and by 1.9 in subsequent years. There was no evidence of decline by 1999, six years after the initial flooding of the reservoir. Because embryonic MeHg burdens from around the experimental reservoir were extremely variable and showed no difference pre- and post-

flooding, higher postflood MeHg burdens in nestling tree swallows were most likely the direct result of changes in MeHg concentrations in their dipteran insect food source.

Although not statistically significant, nestlings raised on the experimental reservoir after flooding had higher MeHg body concentrations than those raised before flooding. These postflood whole body concentrations (109.8-187.9 ng/g dry weight) were lower than most levels (110 ng/g-690 ng/g Hg wet weight) found in four other species of first-year birds (grackles, *Quiscalus quiscula;* starlings, *Sturnus vulgaris;* red-winged blackbirds, *Agelaius phoeniceus;* and cowbirds, *Molothrus aterwhat*) in Maryland (Finley, 1979). Feather MeHg concentrations were higher in postflood nestlings and were within the range of concentrations (median: 1400 ng/g; range: undefined – 1.4 ng/g-wet weight) summarized by Burger (1993) from eight studies based on passerines. It is generally accepted that 5000 ng/g MeHg wet weight in bird feathers is the critical level for toxic effects (Burger and Gochfeld, 1997). All tree swallows that we examined had feather MeHg concentrations well below that level.

MeHg concentrations in liver, muscle, and brain were higher in birds from the experimental reservoir than from the reference lakes. However, all tissue concentrations remained below critical levels known to cause toxic effects. For example, concentrations of MeHg in tree swallow nestlings were similar to those seen in immature insectivorous ducks (common goldeneyes, *Bucephala clangula*) from uncontaminated areas (Fimreite, 1974). Fimreite (1974) found livers in immature common goldeneyes in northwestern Ontario to have between 220-530 ng/g THg wet weight, similar to the mean liver MeHg concentrations of 281.5 ± 9.6 ng/g dry weight in nestlings from the experimental reservoir after flooding. As in feathers, liver Hg concentrations of 5000 ng/g wet weight are generally associated with major toxic effects (Wolfe, 1998). Although there is no

established critical level for MeHg concentrations in muscle, Heinz (Heinz, 1979) associated a decline in reproductive success with concentrations between 670-830 ng/g wet weight in muscle of mallard ducks, much higher than postflood concentrations in tree swallow nestlings in our study (136.7 ± 5.7 ng/g dry weight). Elbert and Anderson (1998) found that the critical brain concentration of MeHg in birds is 500 ng/g wet weight, although other studies have cited slightly higher levels (Wolfe 1998). In passerines, Scheuhammer (1988) reported 25% fatality in zebra finches with brain Hg residues of 20,000 ng/g wet weight, much higher than the 202.3 ± 7.8 ng/g dry weight brain concentrations in tree swallows nesting on the experimental reservoir after flooding.

Although Hg concentrations in tree swallow tissues were below those known to be fatal, relatively little is known about long-term low-level Hg uptake and associated behavioral and physical changes. Heinz (1979) found that females of three generations of mallard ducks fed 500 ng/g MeHg dry weight laid a greater percentage of their eggs outside their nestboxes and laid fewer eggs then control birds. Ducklings from parents fed MeHg were less responsive to maternal calls and were hyper-responsive to fright stimuli relative to ducklings from control broods. Long-term, low-level exposure may also increase susceptibility to infection (Dieter *et al.*, 1983). Spalding *et al.* (1994) examined great white herons (*Ardea herodians*) that were dying of chronic multiple diseases and found elevated Hg body burdens as compared with those birds that were only acutely sick.

Once their plumage has completed development (at about 30 days old), tree swallows must rely on other physiological mechanisms of Hg excretion. If they continue to feed in areas with food containing relatively high MeHg levels, such as those found

around our experimental reservoir, concentrations in their body and tissues will likely continue to increase relatively quickly. The impact of elevated MeHg in young birds and the associated postfledging risks may also be important and should therefore be examined.

Tissue Hg Relationships

Bird feathers are commonly used as a non-invasive method of monitoring MeHg bioaccumulation in bird populations and archived museum specimens (Berg, 1966; Goede and de Bruin, 1984; Lindberg and Odsjo, 1983). Hg that is bound to feathers is stable and resistant to weathering even over long periods of time (Appelquist *et al.*, 1984). In an effort to improve understanding of the relevance of using feather Hg concentrations in assessing levels of Hg toxicity in birds, many studies have examined the relationship between concentrations of Hg in feathers and concentrations of Hg in target tissues such as muscle, liver and brain. Our study is one of only a few, however, that have focused on passerines or immature birds.

The relationship between Hg burdens in embryos and nestlings has also been left relatively unexamined. We found that embryo burdens formed a small percentage of the total nestling MeHg burden after approximately 18 days of growth, and that there was no relationship between concentrations of MeHg in embryos and in tissue of nestlings subsequently collected from the same nestbox. This lack of relationship is most likely due to nestlings receiving the majority of their MeHg burdens from the food they consume and not from their mother. This conclusion is supported by observations of increased rates of Hg methylation and concentrations of MeHg in dipterans in the reservoir throughout the summer.

The relationship between concentrations of MeHg in feathers and body tissues was much stronger than those found between nestlings and embryos. The relationship between feather:liver:muscle Hg concentrations was found to be approximately 7:3:1 ratio wet weight in a variety of other studies (Furness, 1979; Ohlendorf *et al.*, 1985), although Thompson *et al.* (1990) explained in his review that molting pattern and the relative proportion of MeHg to inorganic Hg can complicate this generalization. We found a ratio of 10:2:1 for feather:liver:muscle dry weight MeHg concentrations with some variation between birds. During feather growth, MeHg in the blood readily binds with sulf-hydryl groups in feathers and feathers can act as MeHg sinks that are lost during molt. After feather growth ceases, the blood supply to them atrophies and MeHg can no longer be bound. MeHg then begins to accumulate in body tissues. This phenomenon may explain the proportionately higher concentration of MeHg in nestling bird feathers when compared with feathers in adult birds from other studies. According to Thompson (Thompson *et al.*, 1990), the generalized relationship is best used in species with low Hg burdens where the majority of Hg is MeHg, and when demethylation of MeHg to inorganic Hg in the liver is not important. Here the use of feathers from nestlings of a relatively constant age standardizes for recent dietary intake and molt stage.

In our study, brain concentrations were only weakly correlated with feather concentrations. Elbert (Elbert and Anderson, 1998) found brain concentrations in grebes (*Aechmophorus occidentalis*) to plateau at 335 ng/g. If brain concentrations of MeHg plateau early during nestling growth, we would not expect to find a relationship between brain concentrations and those in feathers, still growing and accumulating MeHg. However, we have no evidence of a plateau of MeHg concentration in the brains of tree swallow nestlings.

Reproductive Success

All tree swallow embryos and nestlings collected at the experimental reservoir and reference lakes had MeHg concentrations below documented critical concentrations for extreme toxicological effects. We also found no obvious negative impacts of elevated MeHg bioaccumulation on the reproductive success and survivorship of the birds at the experimental reservoir. Instead we found that the fitness of birds nesting on the experimental reservoir positively responded to the increase in dipteran food supply after flooding. In particular, postflood clutches were started earlier and had eggs with a slightly larger volume than preflood clutches. Growth rates of wing and bill length were also greater in postflood nestlings compared to those raised at the site prior to flooding. In contrast there was no change in any reproductive parameters in clutches from the reference lakes over the course of the study, although there was also no difference between growth rates in nestlings at the experimental reservoir and those at reference lakes.

It appears that the increase in dipteran insect emergence after creation of the experimental reservoir had a positive effect on female fitness as reflected in certain aspects of egg size. We found that individual egg width and volume were greater in postflood clutches than in preflood. However, the increase in food abundance at our experimental site did not affect total egg volume per clutch, hatchability, or fledging success. Total investment in a clutch most likely reflects the ability of females to overcome the energetic demands of egg production (St. Louis, 1993b). Quinney *et al.* (1986) examined tree swallow populations on three lakes in Ontario with varying degrees of insect abundance, and suggested that food availability had a large impact on the

quality of parental care during the nesting period. Both DeSteven (1978) and Quinney *et al.* (1986) found that female condition had significant effects on egg mass and many studies have linked food abundance with reproductive success (DeSteven, 1978; Wiggins, 1990b).

It has also been suggested that food supply is a cue for the timing of breeding in birds and that laying date is determined by the timing and availability of enough food for females to form eggs (Perrins, 1970). Dipteran insect emergence was much greater on the experimental reservoir in the years after flooding and thus probably affected the clutch initiation date of breeding tree swallows there. In small birds Davies and Lundberg (1985) suggested that overall reproductive success might be limited more by the ability to feed young rather than to form eggs. As a result, clutch initiation date and fledging success may be more affected by food supply than clutch size.

Food supply and parental care also play a large role in nestling growth. Quinney *et al.*(1986) found that tree swallow nestlings raised in regions with abundant food supplies grew more quickly and survived better than those from regions where food was less abundant. In an experimental setting, Boag (1987) found that the quality of food available to nestling zebra finches affected growth rates as well as adult size. This suggests that the increase in food abundance as a result of flooding had a positive affect on the fitness of nestling tree swallows in the area. Our findings of elevated growth rates in wing length and bill length in postflood nestlings as compared with preflood nestlings suggests that the increase in food abundance as a result of flooding had a positive affect on the fitness of nestling tree swallows in the area. However the lack of preflood growth data from reference systems limits our interpretation of these results.

CONCLUSION

This study showed that higher rates of Hg methylation in sediments and flooded vegetation in an experimental reservoir increased MeHg levels in insectivorous songbirds nesting nearby. MeHg concentrations in tree swallows nesting around the experimental reservoir did not reach critical levels and the reproductive success was positively impacted by the increase in food supply. In a similar study on reservoirs in Quebec ranging from 9-11 years old, DesGranges *et al.* (1998) found osprey nesting around reservoirs had much higher Hg concentrations than those nesting in natural habitats, although their overall reproductive success was not impacted. As in our experimental reservoir, reservoirs on the La Grand River stimulated biological productivity and fish populations increased in size. Hg concentrations in Osprey blood were strongly correlated with Hg concentrations in the fish they were eating (DesGranges *et al.*, 1998). Reservoir creation and the subsequent increase in Hg methylation within reservoirs resulted in a transfer of MeHg from the aquatic ecosystem into terrestrial species. Although reservoir creation may result in an increase in food supply for piscivorous and insectivorous species, the impact of moderately elevated MeHg concentrations is still not fully understood.

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